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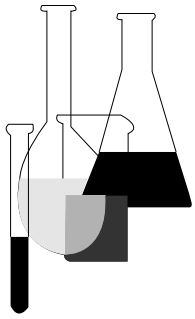
Health Effects Test Guidelines

OPPTS 870.5140
Gene Mutation in
Aspergillus nidulans



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512–0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (http://www.epa.gov/epahome/research.htm) under the heading ''Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines.''

OPPTS 870.5140 Gene mutation in Aspergillus nidulans.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are OPPT 40 CFR 798.5140 Gene mutations in Aspergillus nidulans and OPP 84–2 Mutagenicity Testing (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09–82–025, 1982.

(b) **Purpose.** *Aspergillus nidulans* (*A. nidulans*) is a eukaryotic fungus which has been developed to detect and study a variety of genetic phenomena including chemically induced mutagenesis. *A. nidulans* can be used to detect both forward and reverse gene mutation. These mutations are detected by changes in colonial morphology or nutritional requirements in treated populations. The methionine and 2-thioxanthine forward mutation systems can be used to detect mutations in *A. nidulans.*

(c) **Definitions.** The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this test guideline. The following definition also applies to this test guideline.

Forward mutation is a gene mutation from the wild (parent) type to the mutant condition.

(d) **Reference substances.** These may include, but need not be limited to, ethyl methanesulfonate, cyclophosphamide, or aflatoxin B_1.

(e) **Test method**—(1) **Principle.** Conidia are exposed to the test chemical both with and without metabolic activation and plated on selective medium to determine changes in colonial morphology or nutritional requirements. At the end of a suitable incubation period, mutant colonies are counted and compared to the number of spontaneous mutants in an untreated control culture. Simultaneous determination of survival permits calculation of mutation frequency.

(2) **Description.** Tests for mutation in *A. nidulans* are performed in liquid suspension. Treated conidia are plated on selective medium to determine changes in nutritional requirements or colonial morphology.

(3) **Strain selection**—(i) **Designation.** For the methionine and 2-thioxanthine systems the haploid Glascow *biAl; meth G1* strain is the most commonly used strain although other strains may be appropriate. Any translocation-free strain which produces green colonies on thioxanthine free medium and yellow colonies on medium containing thioxanthine may be used in the thioxanthine system.

(ii) **Preparation and storage.** Stock culture preparation and storage, growth requirements, method of strain identification and demonstration of appropriate phenotypic requirements should be performed using good microbiological techniques and should be documented.

(iii) **Media.** Any medium which supports growth and a characteristic colonial morphology may be used in the assay.

(4) **Preparation of conidia.** Prior to chemical treatment, conidia from four to five single colonies of the appropriate strain are grown at 37 °C on complete medium. At the end of the incubation period, conidia are collected, conidial chains broken up, mycelial debris removed and conidia concentrated prior to removal of the germination inhibitory substance. Germination inhibitory substance should be removed by Tween 80 or diethyl ether.

(5) **Metabolic activation.** Conidia should be exposed to a test substance both in the presence and absence of an appropriate metabolic activation system.

(6) **Control groups.** Concurrent positive and negative (untreated and/or vehicle) controls both with and without metabolic activation should be included in each experiment.

(7) **Test chemicals**—(i) **Vehicle.** Test chemicals and positive control reference substances should be dissolved in an appropriate vehicle and then further diluted in vehicle for use in the assay.

(ii) **Exposure concentrations.** (A) The test should initially be performed over a broad range of concentrations selected on the basis of a preliminary assay. Effective treatment times should also be selected in the preliminary assay.

(B) Each test should include five treatment points, two at fixed concentrations for different time periods, and three at varying concentrations for fixed periods of time.

(C) Among the criteria to be taken into consideration for determining the upper limits of test chemical concentration are cytotoxicity and solubility. Cytotoxicity of the test chemical may be altered in the presence of a metabolic activation system. Relatively insoluble chemicals should be tested up to the limits of solubility. For freely soluble nontoxic chemicals, the upper test chemical concentration should be determined on a case-by-case basis.

(D) When appropriate, a positive response should be confirmed by using a narrow range of test concentrations.

(f) **Test Performance**—(1) **Treatment.** Germinating or quiescent conidia in liquid suspension should be exposed to the test chemical at

37 °C under conditions of yellow light and controlled pH and oxygen tension. At the end of the exposure period, treatment should be terminated by repeated centrifugation and washing of the conidia or by dilution. Chemical neutralization of the test agent may also be used but is not recommended.

(2) **Media**—(i) **Methionine system.** For the methionine system, conidia should be plated on methionine deficient medium for mutant selection and on medium supplemented with methionine to determine survival.

(ii) **Thioxanthine system.** (A) For the 2-thioxanthine system, treated conidia should be plated on nitrogen-free glucose and salts minimal medium containing 2-thioxanthine.

(B) After incubation, green colonies should be counted and isolated by restreaking. The isolated colonies should be classified on the basis of genetic criteria. Yellow, wild-type colonies will grow on the same plate. This permits concurrent determination of survival and an estimation of mutation frequency.

(3) **Determination of mutation frequency and viability.** In both systems, mutation frequency and viability should be determined immediately before and immediately after chemical treatment.

(4) **Incubation conditions.** All incubations should be at 37 °C. Incubation time will vary depending upon system and endpoint (mutation or viability) being determined.

(5) **Number of cultures.** (i) At least 10 independent plates per concentration with no more than 20 colonies per plate should be used in the methionine system.

(ii) Fifteen to twenty plates per concentration are preferred for the 2-thioxanthine system.

(g) **Data and report**—(1) **Treatment of results.** Individual plate counts for test substance and controls should be presented for both mutation induction and survival. The mean number of colonies per plate and standard deviation should also be presented. Data should be presented in tabular form indicating, as applicable, numbers of colonies counted, and numbers and classification of mutants identified. Sufficient detail should be provided for verification of survival and mutation frequencies.

(2) **Statistical evaluation.** Data should be evaluated by appropriate statistical methods.

(3) **Interpretation of results.** (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of mutant colonies. Another criterion may

be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of mutant colonies or a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) **Test evaluation.** (i) Positive results from the methionine and 2-thioxanthine systems in *A. nidulans* indicate that, under the test conditions, the test substance causes gene (point) mutations in the DNA of this organism caused by base-pair changes and small deletions in the genome.

(ii) Negative results indicate that under the test conditions the test chemical is not mutagenic in *A. nidulans*.

(5) **Test report.** In addition to the reporting recommendations as specified under 40 CFR part 792, subpart J, the following specific information should be reported:

(i) Strain of organism used in the assay.

(ii) Test chemical vehicle, doses used, rationale for dose selection, and toxicity data.

(iii) Method used for preparation of conidia.

(iv) Treatment conditions, including length of exposure and method used to stop treatment.

(v) Details of both the protocol used to prepare the metabolic activation system and of its use in the assay.

(vi) Incubation times and temperature.

(vii) Positive and negative controls.

(viii) Dose-response relationship, if applicable.

(h) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Ames, B.N. et al. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutation Research* 31:347-364 (1975).

(2) Kafer, E. et al. *Aspergillus nidulans*: systems and results of tests for chemical induction of mitotic segregation and mutation. I. Diploid and

duplication assay systems: a report of the U.S. EPA's Gene-Tox Program, *Mutation Research* 98:1–48 (1982).

(3) Munson, R.J. and Goodhead, D.T. Relation between induced mutation frequency and cell survival: a theoretical approach and an examination of experimental data for eukaryotes. *Mutation Research* 42:145–159 (1977).

(4) Scott, B.R. et al. *Aspergillus nidulans*: systems and results of tests for mitotic segregation and mutation. II. Haploid assay systems and overall response of all systems: a report of the U.S. EPA's Gene-Tox Program. *Mutation Research* 98:49–94 (1982).